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Original article

Fasting and post-oral-glucose-load levels of methylglyoxal are associated with microvascular, but not macrovascular, disease in individuals with and without (pre)diabetes: The Maastricht Study

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ABSTRACT

Aims. – Reactive dicarbonyl compounds, such as methylglyoxal (MGO), rise during an oral glucose tolerance test (OGTT), particularly in (pre)diabetes. Fasting MGO levels are associated with chronic kidney disease (CKD) and cardiovascular disease (CVD) in patients with poorly controlled type 2 diabetes mellitus (T2DM). Yet, whether fasting or post-OGTT plasma MGO levels are associated with vascular disease in people with (pre)diabetes is unknown.

Methods. – Subjects with normal glucose metabolism ($n = 1796$; age: 57.9 ± 8.2 years; 43.3% men), prediabetes ($n = 478$; age: 61.6 ± 7.6 years; 54.0% men) and T2DM ($n = 669$; age: 63.0 ± 7.5 years; 67.0% men) from the Maastricht Study underwent OGTTs. Plasma MGO levels were measured at baseline and 2 h after OGTT by mass spectrometry. Prior CVD was established *via* questionnaire. CKD was reflected by estimated glomerular filtration rate (eGFR) and albuminuria; retinopathy was assessed using retinal photographs. Data were analyzed using logistic regression adjusted for gender, age, smoking, systolic blood pressure, total-to-HDL cholesterol ratio, triglycerides, HbA_{1c}, BMI and medication use. Odd ratios (ORs) were expressed per standard deviation of LN-transformed MGO.

Results. – Fasting and post-OGTT MGO levels were associated with higher ORs for albuminuria $\geq$ 30 mg/24 h [fasting: 1.12 (95% CI: 0.97–1.29); post-OGTT: 1.19 (1.01–1.41)], eGFR $<$ 60 mL/min/1.73 m^2 [fasting: 1.58 (95% CI: 1.38–1.82), post-OGTT: 1.57 (1.34–1.83)] and retinopathy [fasting: 1.59 (95% CI: 1.01–2.53), post-OGTT: 1.38 (0.77–2.48)]. No associations with prior CVD were found.

Conclusion. – Fasting and post-OGTT MGO levels were associated with microvascular disease, but not prior CVD. Thus, therapeutic strategies directed at lowering MGO levels may prevent microvascular disease.

Introduction

Type 2 diabetes mellitus (T2DM) remains a leading risk factor for macro- and microvascular diseases, such as cardiovascular disease (CVD) [1], chronic kidney disease (CKD) and blindness. A key mediator in the association between diabetes and vascular disease may be the formation of dicarbonyl compounds, reactive glucose metabolites that interact with proteins to form advanced

Abbreviations: 3-DG, 3-deoxyglucosone; AGEs, advanced glycation end-products; BMI, body mass index; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; GO, glyoxal; OGTT, oral glucose tolerance test; HDL, high-density lipoprotein; MGO, methylglyoxal; UPLC-MS/MS, ultra-high-performance liquid chromatography/tandem mass spectrometry; UAE, urinary albumin excretion.

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glycation end-products (AGEs) [2]. Methylglyoxal (MGO), glyoxal (GO) and 3-deoxyglucosone (3-DG) have been identified as major dicarbonyl compounds [3]. Of these, MGO has proved to be the most reactive dicarbonyl, and is a potential driver of diabetes complications [4,5] and CVD [6]. Dicarbonyls can damage endothelium, leading to prolonged endothelial activation and impaired vasorelaxation [5]. Indeed, it was previously shown that fasting plasma MGO levels are associated with plasma markers of microvascular endothelial dysfunction (ED) in patients with type 1 diabetes (T1DM) [7]. Moreover, higher levels of fasting plasma MGO are associated with incident CVD and prevalent CKD in patients with either T1DM [7] or T2DM [8]. However, it remains unclear whether these findings extend to prediabetes and normal glucose metabolism.

Previously, it was found that plasma dicarbonyls increase with glucose excursions after an oral glucose tolerance test (OGTT) or a mixed meal [9]. As post-OGTT plasma glucose levels are more strongly associated with incident CVD than are fasting plasma glucose levels [10], our hypothesis was that post-load rather than fasting levels of MGO might also be more strongly associated with vascular outcomes. These considerations are important as peak MGO levels can be reduced by interventions that curtail (postprandial) glucose excursions [9] and compounds with MGO-quenching properties, such as pyridoxamine [11], and carnosine or its analogues [12,13].

Therefore, the present study investigated, in subjects with NGM, prediabetes and T2DM, and the cross-sectional associations of fasting and post-OGTT plasma levels of MGO, and two other major dicarbonyls (GO and 3-DG), with CVD and two markers of subclinical macrovascular disease–the ankle–brachial index (ABI) and carotid intima–media thickness (cIMT)–as well as associations with microvascular disease (CKD, retinopathy) and markers of microvascular endothelial dysfunction (plasma microvascular endothelial activation score, retinal arteriolar and venular dilatation, heat-induced skin hyperaemia).

Methods

Study population

Data from the Maastricht Study, an observational prospective population-based cohort study, were enriched by including patients with T2DM. The rationale and design of that study have been described previously [14]. In brief, the study focuses on the aetiology, pathophysiology, complications and comorbidities of T2DM, and is characterized by an extensive phenotyping approach. Those eligible for participation were all individuals aged 40 to 75 years living in the southern part of the Netherlands. Participants were recruited through mass media campaigns, municipal registries and regional diabetes patient registries *via* mailings. Recruitment was stratified according to known T2DM status, with an oversampling of patients with T2DM for reasons of efficiency. The present report includes data from 3451 participants who completed the baseline survey between November 2010 and September 2013; examinations of each participant were performed within a time window of 3 months.

The study was approved by the institutional medical ethics committee (NL31329.068.10) and Minister of Health, Welfare and Sport of the Netherlands (permit number: 131088-105234-PG). All participants gave their written informed consent.

Oral glucose tolerance tests

All subjects underwent a 75-g 2-h OGTT after an overnight fast, except for those taking insulin or with a fasting glucose level > 11.0 mmol/L (as determined by finger-prick test) for safety reasons. For all included subjects, fasting glucose levels and information on their diabetes medications were used to determine their glucose metabolic status. This status was defined according to World Health Organization 2006 criteria as either normal glucose metabolism (NGM), impaired fasting glucose (IFG) and impaired glucose tolerance (IGT), which were combined as impaired glucose metabolism (IGM), also known as prediabetes, and T2DM.

Dicarbonyl levels and other measurements

Plasma levels of MGO, GO and 3-DG were measured by ultra-high-performance liquid chromatography and tandem mass spectrometry (UPLC-MS/MS) [15] at T = 0 min and T = 120 min during OGTT. In brief, ethylenediaminetetraacetic acid (EDTA) plasma samples (25 μL) were mixed with 75 μL of d_8-*O*-phenylenediamine (oPD; 10 mg of oPD in 10 mL of 1.6 mol/L perchloric acid) in an Eppendorf cup. After overnight (20 h) reaction at room temperature and shielded from light, 10 μL of internal standard stock solution was added. Samples were mixed and subsequently centrifuged for 20 min at 21,000 g at a temperature of 4 °C; 10 μL was injected for UPLC-MS/MS analysis. Plasma samples were then stored at -80 °C until needed for analysis. Interassay variations for MGO, GO and 3-DG were 4.3%, 5.1% and 2.2%, respectively [15].

Prior CVD and markers of macrovascular disease

Presence of CVD was assessed by self-reported questionnaire [14]. The VP-2000 vascular system (Omron, Kyoto, Japan) was used to determine the ABI by measuring systolic blood pressure at the brachial artery of the left and right arms as well as above the left and right ankle joints [14]. Values in the leg with the lowest available index were also included. An ABI < 0.9 was used as the cut-off value indicative of peripheral arterial disease [16]. The cIMT was measured as described previously elsewhere [17], and the structural properties of the carotid artery were determined using an ultrasound scanner equipped with a 7.5-MHz linear probe (MyLab 70, Esaote Europe B.V., Maastricht, The Netherlands).

Markers of CKD

In addition, 24-h urinary albumin excretion (UAE) was measured twice: UAE values 30–300 mg/24 h indicated microalbuminuria, whereas > 300 mg/24 h indicated macroalbuminuria. For our main analyses of albuminuria, micro- and macroalbuminuria were combined. The estimated glomerular filtration rate (eGFR) was calculated (in mL/min/1.73 m^2) using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation based on both serum creatinine and serum cystatin C [18].

Retinopathy

The presence of retinopathy was assessed in both eyes by fundus photographs taken with an automated fundus camera (AFC-230 digital retinal camera; Nidek Co., Ltd., Gamagori, Aichi, Japan) [14]. Retinopathy was graded according to the diabetic retinopathy (DR) disease severity scale and International Clinical DR Disease Severity Scale (American Academy of Ophthalmology Retina/Vitreous Panel, 2014).

Measurements of microvascular endothelial dysfunction

Retinal arteriolar and venular dilatation responses to flicker pupil light, which are thought to be related to nutritional demands of activated retinal neurons, were measured in a dimly lit room

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using a dynamic vessel analyzer (IMEDOS Systems GmbH, Jena, Germany). For each participant, either the left or right eye was randomly measured. The percentage dilatation over baseline was based on the average dilatation achieved at 10-s and 40-s time points during the flicker stimulation period [19].

The heat-induced skin hyperaemic response, thought to be related to skin metabolic and thermoregulatory function, is considered a marker of endothelial microvascular function. Skin blood flow was measured, as described previously, by means of laser Doppler velocimetry (PeriFlux 5000; Perimed AB, Järfälla, Sweden), using a thermostatic laser Doppler probe (PF457; Perimed) on the dorsal side of the left wrist. The heat-induced skin hyperaemic response was expressed as the percentage increase in average perfusion units over the average baseline perfusion units during the 23-min heating phase [19].

As described previously elsewhere [20], plasma biomarkers of microvascular ED [soluble vascular cell adhesion molecule-1 (sVCAM-1), soluble intercellular adhesion molecule (sICAM-1), soluble E-selectin (sE-selectin), von Willebrand factor (vWF)] were measured, the former three biomarkers in EDTA plasma samples using commercially available 4-plex sandwich immunoassay kits (Meso Scale Diagnostics LLC, Rockville, MD, USA), and the latter (vWF) in citrated plasma using sandwich enzyme-linked immunosorbent assay (ELISA; DAKO, Jena, Germany). For the present study, intra- and interassay coefficients of variation were 3.5% and 5.9% for sVCAM-1, 2.5% and 5.3% for sICAM-1, 6.4% and 6.0% for sE-selectin, and 3.2% and 5.4% for vWF. These markers were combined as a composite Z-score for our primary analyses.

Measurement of potential confounders

Weight, height, body mass index (BMI), blood pressure, plasma glucose levels, glycohaemoglobin A1c (HbA_{1c}) and plasma lipid profiles were measured as described elsewhere [14]. Smoking status (never, former, current) was assessed by questionnaire, and the use of lipid-modifying, antihypertensive and glucose-lowering medications was assessed during a medication interview.

Selection of study participants for analysis

For the present study analyses, data from the first 3451 participants of the Maastricht Study were used, after first excluding participants with either T1DM ($n = 37$) or diabetes of undetermined type ($n = 4$). Next were excluded those individuals for whom plasma samples to measure dicarbonyls were not available at either T = 0 min ($n = 121$) or T = 120 min ($n = 393$) of OGTT and those with missing confounders ($n = 50$), thereby arriving at 2943 participants (Fig. 1). However, for logistical reasons, not all outcomes could be assessed in all participants. Prior CVD was only available for 2920 participants, ABI for 2720, cIMT for 2503, albuminuria for 2919, eGFR for 2943, retinopathy for 2442, retinal arteriolar average dilatation for 1985, retinal venular average dilatation for 2017, heat-induced skin hyperaemia for 1419 and plasma ED scores for 2924.

Statistical analyses

All analyses were performed with SPSS version 20.0 software for Windows (IBM Corp., Armonk, NY, USA). Plasma dicarbonyl and glucose levels, UAE and heat-induced skin hyperaemia were LN-transformed prior to analyses to reduce the influence of potential outliers. Logistic regression models were used to investigate cross-sectional associations between plasma dicarbonyl and glucose levels and the presence of CVD, ABI < 0.9, markers indicative of CKD and retinopathy. In particular, ABI sensitivity analyses were performed, excluding those with scores ≥ 1.3 ($n = 167$) or ≥ 1.4 ($n = 17$), as such ABI values can be indicative of vascular calcification [16]. Linear regression models were also applied to investigate associations between fasting and post-load plasma Z-scores for plasma dicarbonyl and glucose levels, as well as cIMT, ABI, UAE, eGFR and markers of microvascular ED on a continuous scale. All models were adjusted for gender, age and glucose metabolic status (model 1), with further adjustments performed for cardiovascular risk factors: BMI, systolic blood pressure, total-to-high-density lipoprotein cholesterol (HDL-C) ratio, triglycerides, smoking status, and lipid-modifying, antihypertensive and glucose-lowering treatments (model 2). Finally, whether glycaemic control influenced these associations was investigated after adjusting model 2 for HbA_{1c} levels (model 3).

Interaction analyses also evaluated whether any of the associations found differed between men and women (gender × MGO, GO or 3-DG) or according to glucose metabolic status (IGM or T2DM × MGO, GO or 3-DG) by adding interaction terms to model 3. $P_{interaction} < 0.10$ was considered statistically significant for these analyses.

Results

Table I presents the clinical characteristics of the Maastricht Study participants included in the present analyses according to glucose metabolic status.

Fasting and post-OGTT dicarbonyl levels

Compared with NGM, fasting plasma MGO levels were higher in prediabetes and T2DM subjects ($P_{trend} < 0.05$); this difference appeared to be more evident after OGTT at T = 120 min (Table I). However, no increase in MGO levels was observed after OGTT in those with NGM (Δ -45.0 ± 99.0 nmol/L) or prediabetes (Δ -33.0 ± 99.8 nmol/L), whereas post-OGTT plasma MGO levels were increased in T2DM patients (Δ 22.4 ± 133.8 nmol/L; Table I). In addition, plasma GO levels were significantly higher in those with prediabetes and T2DM after OGTT, but not at baseline (Table 1), whereas 3-DG and glucose levels were increased in these subjects both before and after OGTT (Table 1).

Fasting and post-OGTT plasma dicarbonyls and prior CVD, ABI and cIMT

On first investigating the associations between fasting (T = 0 min) and post-OGTT (T = 120 min) plasma levels of MGO, GO and 3-DG and the presence of CVD and subclinical markers of macrovascular disease (ABI, cIMT), no consistent associations were found overall between fasting and post-OGTT plasma levels of MGO, GO and 3-DG and either prior CVD, ABI (cut-off point of < 0.9 or continuous scale) or cIMT (Table 2). When those with an ABI ≥ 1.4 or ≥ 1.3 were excluded, the associations between plasma dicarbonyls and ABI did not change (data not shown). In addition, no consistent interactions were found for either gender or glucose metabolic status (data not shown).

Fasting and post-OGTT plasma dicarbonyls and presence of albuminuria, reduced eGFR and retinopathy

Higher fasting plasma MGO levels were associated with greater odds of having macroalbuminuria independently of gender, age and glucose metabolic status (Table 3, model 1). This association was not attenuated after further adjusting for total-to-HDL-C ratio, triglycerides, lipid-modifying treatment, smoking status, systolic blood pressure, BMI, and blood pressure-lowering and glucose-lowering treatments (Table 3, model 2) or HbA_{1c} (Table 3, model 3).

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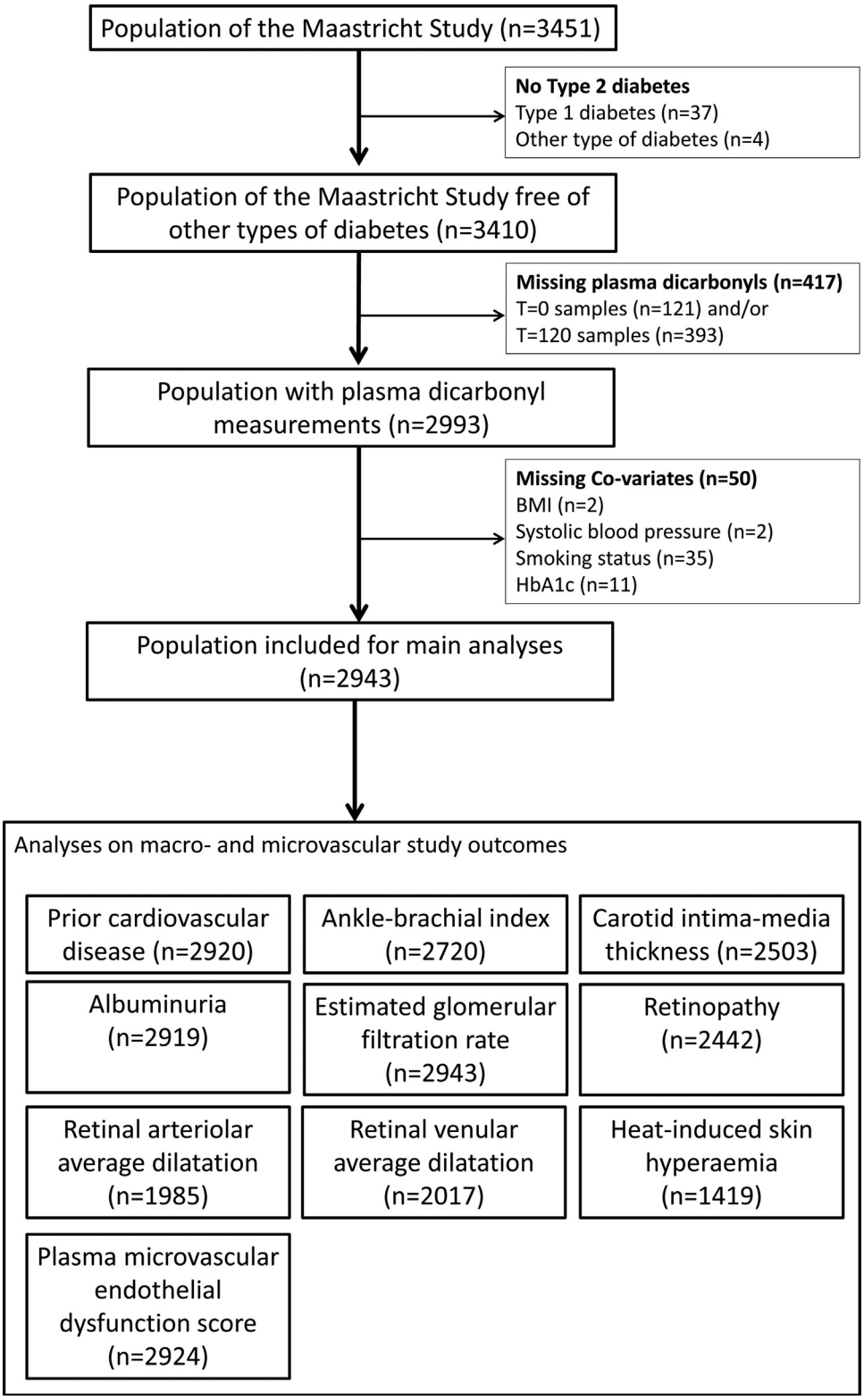

Fig. 1. Flow chart of the study population-selection process.

As for post-OGTT plasma levels of MGO, a similar association was found with macroalbuminuria as with fasting plasma MGO levels in model 1, which was attenuated and no longer statistically significant after additional adjustments (Table 3, models 2–3). Moreover, similar but weaker associations were found between fasting plasma MGO levels and albuminuria (Table 3, model 1), which were attenuated and of borderline significance after further model adjustments (Table 3, models 2–3). On the other hand, associations between post-OGTT plasma MGO levels and greater odds of having albuminuria remained statistically significant throughout all statistical models (Table 3, models 1–3). However, when additional adjustments were made for the association between post-OGTT plasma MGO levels and albuminuria for fasting MGO levels, the point estimate of this association appeared

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Table 1
Participant characteristics stratified according to glucose metabolism status.

	NGM ($n = 1796$)	Prediabetes ($n = 478$)	T2DM ($n = 669$)	P_{trend}
Age (years)	57.9 ± 8.2	61.6 ± 7.6	63.0 ± 7.5	< 0.05
Gender (male, %)	43.3	54.0	67.0	< 0.05
HbA_{1c} (%)	5.4 ± 0.3	5.7 ± 0.4	6.6 ± 0.6	< 0.05
HbA_{1c} (mmol/mol)	36.1 ± 3.8	38.8 ± 4.4	48.4 ± 7.0	< 0.05
Body mass index (kg/m^2)	25.6 ± 3.6	27.7 ± 4.2	29.5 ± 4.8	< 0.05
Total cholesterol (mmol/L)	5.6 ± 1.0	5.4 ± 1.1	4.5 ± 1.0	< 0.05
HDL cholesterol (mmol/L)	1.6 ± 0.5	1.5 ± 0.4	1.3 ± 0.4	< 0.05
Triglycerides (mmol/L)	1.1 (0.8–1.5)	1.4 (1.0–1.8)	1.5 (1.2–2.1)	< 0.05
Systolic blood pressure (mmHg)	130.7 ± 17.3	137.2 ± 17.0	141.4 ± 17.4	< 0.05
Diastolic blood pressure (mmHg)	78.1 ± 9.6	78.1 ± 9.6	77.8 ± 9.5	< 0.05
Smoking status [never, ex, current (%)]	(39.2, 48.2, 12.6)	(30.3, 57.3, 12.3)	(29.7, 56.2, 14.1)	< 0.05
Glucose-lowering treatment (%)	0	0	71.4	< 0.05
Lipid-lowering treatment (%)	17.2	34.9	71.6	< 0.05
Antihypertensive medication (%)	22.5	44.8	68.8	< 0.05
Oral glucose tolerance test				
T = 0 min glucose (mmol/L)	5.2 (4.9–5.5)	6.1 (5.5–6.4)	7.3 (6.6–8.1)	< 0.05
T = 120 min glucose (mmol/L)	5.4 (4.6–6.2)	8.4 (7.2–9.4)	14.4 (11.9–17.2)	< 0.05
Δ Glucose (mmol/L)	0.2 ± 1.1	2.2 ± 2.0	7.0 ± 3.4	< 0.05
T = 0 min MGO (nmol/L)	308.3 (269.5–358.1)	325.5 (283.6–383.5)	346.0 (300.4–399.8)	< 0.05
T = 120 min MGO (nmol/L)	262.3 (229.0–305.7)	298.2 (259.7–343.5)	366.2 (319.7–437.0)	< 0.05
Δ MGO (nmol/L)	−45.77 ± 99.1	−33.4 ± 99.8	22.4 ± 133.8	< 0.05
T = 0 min GO (nmol/L)	1216.6 (1018.0–1476.5)	1209.3 (1025.6–1455.8)	1182.8 (1007.5–1488.9)	0.75
T = 120 min GO (nmol/L)	1159.0 (976.0–1402.3)	1246.8 (1058.5–1463.1)	1476.6 (1246.4–1749.5)	< 0.05
Δ GO (nmol/L)	−56.3 ± 538.1	2.5 ± 562.9	281.5 ± 663.0	< 0.05
T = 0 min 3-DG (nmol/L)	1141.9 (1044.0–1263.6)	1295.4 (1167.6–1468.6)	1630.2 (1454.2–1886.0)	< 0.05
T = 120 min 3-DG (nmol/L)	1190.9 (1014.7–1370.5)	1733.0 (1483.5–1999.3)	2891.5 (2416.1–3389.3)	< 0.05
Δ 3-DG (nmol/L)	43.7 ± 260.0	402.5 ± 378.2	1213.8 ± 617.0	< 0.05
Macrovascular disease markers				
Previous CVD (%)	12.0	14.0	25.3	< 0.05
Ankle–brachial index (ABI)	1.2 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	< 0.05
ABI < 0.9 (%)	1.3	1.6	3.9	< 0.05
cIMT (μm)	842.5 ± 148.3	872.6 ± 157.3	890.1 ± 172.2	< 0.05
Microvascular disease markers				
Presence of retinopathy (%)	0.1	0.3	1.9	< 0.05
eGFR (mL/min/1.73 m^2)	80.5 ± 14.1	79.6 ± 14.1	81.6 ± 18.0	0.17
eGFR < 60 mL/min/1.73 m^2 (%)	5.4	7.1	10.0	< 0.05
UAE (mg/mmol/24 h)	0.5 (0.2–0.8)	0.5 (0.3–0.8)	0.7 (0.4–1.3)	< 0.05
Microalbuminuria (%)	3.7	5.9	14.3	< 0.05
Macroalbuminuria (%)	0.4	0.8	0.9	< 0.05
Microvascular endothelial dysfunction markers				
Flicker pupil light-induced arteriolar dilatation (%)	3.4 ± 2.8	3.0 ± 2.8	2.5 ± 2.6	< 0.05
Flicker pupil light-induced venular dilatation (%)	3.9 ± 2.2	4.0 ± 2.2	3.7 ± 2.2	0.15
Heat-induced skin hyperaemia (%)	1085.8 (639.3–1618.8)	995.4 (575.7–1541.6)	824.6 (517.3–1249.2)	< 0.05
von Willebrand factor (%)	125.4 ± 44.2	136.4 ± 48.1	140.5 ± 52.0	< 0.05
sE-selectin (ng/mL)	102.1 ± 46.7	118.8 ± 55.9	143.7 ± 74.2	< 0.05
sICAM (ng/mL)	335.4 ± 79.3	360.7 ± 97.9	380.7 ± 111.7	< 0.05
sVCAM (ng/mL)	413.1 ± 86.5	424.2 ± 103.4	447.3 ± 107.3	< 0.05
Plasma microvascular score (Z-score)	−0.2 ± 0.8	0.1 ± 1.1	0.5 ± 1.2	< 0.05

Data are expressed as means ± standard deviation, interquartile ranges or percentages as appropriate; P_{trend} was calculated by linear regression or Chi^2 analyses; skewed variables were LN-normalized prior to analyses; NGM: normal glucose metabolism; T2DM: type 2 diabetes mellitus; HbA_{1c}: glycated haemoglobin; HDL: high-density lipoprotein; MGO: methylglyoxal; GO: glyoxal; 3-DG: 3-deoxyglucosone; CVD: cardiovascular disease; cIMT: carotid intima–media thickness; eGFR: estimated glomerular filtration rate; UAE: urinary albumin excretion; sE-selectin: soluble E-selectin; sICAM: soluble intercellular adhesion molecule; sVCAM: soluble vascular adhesion molecule

to be slightly attenuated and was no longer statistically significant [OR: 1.16 (95% CI: 0.96–1.39) vs. 1.19 (1.01–1.41)]. Higher fasting and post-OGTT plasma MGO levels were also associated with higher UAE when analyzed on a continuous scale (Table S1; see supplementary materials associated with this material online).

In line with these findings, higher fasting plasma MGO levels were associated with greater odds of having a lower eGFR (< 60 mL/min/1.73 m^2) after adjusting for gender, age and glucose metabolic status (Table 3, model 1), an association that was not attenuated when the model was further adjusted (Table 3, models 2–3). Point estimates for these associations were similar for post-OGTT plasma MGO levels (Table 3, models 1–3). However, after adjusting for the association between post-OGTT plasma MGO levels and a decreased eGFR with fasting MGO levels, it was found that the point estimate weakened [OR: 1.34 (95% CI: 1.12–1.60) vs. 1.57 (1.34–1.83)]. Higher fasting and post-OGTT plasma MGO levels were also significantly associated with a lower eGFR when analyzed on a continuous scale (Table S1).

Even though retinopathy was numerically infrequent ($n = 14$ cases), our study found that higher fasting plasma MGO levels were associated with greater odds of having retinopathy, which became statistically significant in the fully adjusted model (Table 3, model 3). As for post-OGTT plasma MGO levels, point estimates of these associations indicated a similar direction, but were somewhat weaker and not statistically significant (Table 3, models 1–3).

As regards either fasting or post-OGTT plasma levels of GO, no significant associations were found with the presence of albuminuria, eGFR < 60 mL/min/1.73 m^2 or retinopathy. However, an association was revealed between higher fasting, but not post-OGTT, plasma levels of GO and a lower eGFR when analyzed on a continuous scale (Table S1).

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Table 2
Associations between plasma dicarbonyl levels and markers of macrovascular disease.

	Model	Previous CVD ($n = 448$)	ABI < 0.9 ($n = 52$)	ABI (on continuous scale)	cIMT
MGO T = 0 min	1	1.00 (0.90–1.11)	0.78 (0.57–1.06)	−0.03 (−0.07 to 0.01)	0.02 (−0.02 to 0.05)
	2	0.99 (0.89–1.11)	0.75 (0.55–1.02)	−0.02 (−0.05 to 0.02)	0.01 (−0.03 to 0.05)
	3	0.99 (0.88–1.11)	0.73 (0.53–1.01)	−0.01 (−0.05 to 0.03)	0.01 (−0.03 to 0.04)
MGO T = 120 min	1	0.98 (0.87–1.11)	0.83 (0.59–1.15)	−0.03 (−0.07 to 0.01)	0.01 (−0.03 to 0.05)
	2	0.99 (0.87–1.12)	0.79 (0.56–1.12)	−0.02 (−0.06 to 0.02)	0.00 (−0.04 to 0.04)
	3	0.98 (0.86–1.11)	0.76 (0.53–1.09)	−0.01 (−0.06 to 0.03)	0.00 (−0.05 to 0.04)
GO T = 0 min	1	0.90 (0.81–1.00)	0.99 (0.75–1.30)	0.01 (−0.03 to 0.05)	0.01 (−0.02 to 0.05)
	2	0.95 (0.85–1.06)	1.05 (0.80–1.38)	−0.01 (−0.05 to 0.03)	0.02 (−0.02 to 0.05)
	3	0.95 (0.85–1.06)	1.05 (0.80–1.38)	−0.01 (−0.05 to 0.03)	0.02 (−0.02 to 0.05)
GO T = 120 min	1	0.90 (0.80–1.00)	0.76 (0.57–1.03)	0.06 (0.02 to 0.09)	0.00 (−0.04 to 0.04)
	2	0.96 (0.86–1.08)	0.81 (0.60–1.09)	0.03 (−0.01 to 0.07)	0.00 (−0.04 to 0.04)
	3	0.96 (0.86–1.08)	0.81 (0.60–1.09)	0.03 (−0.01 to 0.07)	0.00 (−0.04 to 0.04)
3-DG T = 0 min	1	1.06 (0.92–1.22)	0.89 (0.62–1.30)	−0.05 (−0.10 to 0.00)	0.01 (−0.04 to 0.06)
	2	1.01 (0.87–1.18)	0.79 (0.53–1.18)	−0.03 (−0.08 to 0.03)	0.00 (−0.05 to 0.05)
	3	0.99 (0.84–1.16)	0.74 (0.49–1.13)	−0.01 (−0.07 to 0.04)	−0.01 (−0.07 to 0.05)
3-DG T = 120 min	1	0.99 (0.83–1.18)	0.87 (0.56–1.36)	−0.05 (−0.11 to 0.02)	−0.01 (−0.08 to 0.05)
	2	0.92 (0.76–1.12)	0.71 (0.44–1.17)	−0.02 (−0.08 to 0.05)	−0.04 (−0.10 to 0.03)
	3	0.89 (0.73–1.10)	0.69 (0.40–1.11)	0.00 (−0.07 to 0.07)	−0.05 (−0.11 to 0.02)
Glucose T = 0 min	1	0.87 (0.73–1.02)	1.13 (0.75–1.72)	−0.02 (−0.09 to 0.04)	0.01 (−0.06 to 0.07)
	2	0.81 (0.68–0.97)	1.06 (0.69–1.64)	0.00 (−0.07 to 0.06)	0.00 (−0.06 to 0.07)
	3	0.75 (0.62–0.91)	1.02 (0.63–1.64)	0.03 (−0.04 to 0.10)	−0.01 (−0.08 to 0.06)
Glucose T = 120 min	1	0.93 (0.77–1.12)	0.95 (0.59–1.53)	−0.02 (−0.09 to 0.05)	−0.02 (−0.09 to 0.05)
	2	0.87 (0.71–1.07)	0.82 (0.49–1.37)	0.00 (−0.07 to 0.07)	−0.04 (−0.11 to 0.03)
	3	0.85 (0.69–1.04)	0.77 (0.45–1.33)	0.02 (−0.06 to 0.09)	−0.05 (−0.12 to 0.03)

Data analysis by logistic [previous CVD, ankle–brachial index (ABI) < 0.9] or linear [ABI, carotid intima–media thickness (cIMT)] regression, expressed as odds ratios (previous CVD, ABI < 0.9) or 1 standard deviation (SD) difference (cIMT, ABI) per SD increase of independent variable; plasma dicarbonyl and glucose levels LN-transformed prior to analyses; Model 1: adjusted for age, gender, presence of impaired glucose metabolism or type 2 diabetes mellitus; Model 2: model 1 + adjusted for total-to-HDL-C ratio, triglycerides, lipid-modifying treatment, smoking status, systolic blood pressure, blood-pressure-lowering treatment, body mass index, glucose-lowering treatment; Model 3: model 2 + adjusted for HbA_{1c}; CVD: cardiovascular disease; MGO: methylglyoxal; GO: glyoxal; 3-DG: 3-deoxyglucosone; HDL-C: high-density lipoprotein cholesterol.

Table 3
Associations between plasma levels of dicarbonyl and markers of microvascular disease.

	Model	Macroalbuminuria (> 300 mg/24 h) ($n = 17$ cases)	Albuminuria (>30 mg/24 h) ($n = 211$ cases)	eGFR (< 60 mL/min/1.73 m^2) ($n = 197$ cases)	Retinopathy ($n = 14$ cases)
MGO T = 0 min	1	1.57 (1.14–2.17)	1.15 (1.00–1.32)	1.57 (1.37–1.79)	1.37 (0.95–1.96)
	2	1.63 (1.11–2.39)	1.13 (0.98–1.30)	1.57 (1.36–1.80)	1.54 (0.98–2.44)
	3	1.61 (1.10–2.38)	1.12 (0.97–1.29)	1.58 (1.38–1.82)	1.60 (1.01–2.53)
MGO T = 120 min	1	1.55 (1.00–2.40)	1.22 (1.04–1.42)	1.54 (1.32–1.79)	1.26 (0.73–2.17)
	2	1.46 (0.95–2.26)	1.21 (1.02–1.42)	1.54 (1.31–1.80)	1.25 (0.69–2.25)
	3	1.41 (0.90–2.21)	1.19 (1.01–1.41)	1.57 (1.34–1.83)	1.38 (0.77–2.48)
GO T = 0 min	1	1.05 (0.65–1.68)	1.01 (0.88–1.17)	1.03 (0.89–1.20)	1.51 (0.96–2.38)
	2	1.09 (0.67–1.76)	1.02 (0.89–1.18)	1.07 (0.92–1.24)	1.53 (0.92–2.54)
	3	1.08 (0.66–1.77)	1.02 (0.89–1.18)	1.07 (0.92–1.24)	1.57 (0.95–2.59)
GO T = 120 min	1	1.18 (0.72–1.92)	1.05 (0.90–1.23)	1.00 (0.85–1.17)	1.17 (0.68–1.99)
	2	1.19 (0.72–1.97)	1.06 (0.91–1.24)	1.04 (0.89–1.22)	1.08 (0.59–1.98)
	3	1.15 (0.70–1.91)	1.06 (0.90–1.23)	1.04 (0.89–1.23)	1.16 (0.63–2.13)
3-DG T = 0 min	1	2.22 (1.18–4.18)	1.16 (0.96–1.41)	1.07 (0.87–1.31)	0.90 (0.47–1.70)
	2	1.96 (1.01–3.82)	1.12 (0.92–1.37)	1.01 (0.81–1.24)	0.78 (0.40–1.52)
	3	1.84 (0.90–3.74)	1.09 (0.88–1.35)	1.02 (0.82–1.28)	0.88 (0.43–1.81)
3-DG T = 120 min	1	3.77 (1.60–8.84)	1.23 (0.96–1.59)	0.91 (0.71–1.18)	1.41 (0.56–3.52)
	2	3.57 (1.30–9.80)	1.15 (0.88–1.51)	0.82 (0.62–1.07)	1.13 (0.45–2.87)
	3	3.36 (1.18–9.58)	1.11 (0.84–1.48)	0.82 (0.62–1.08)	1.48 (0.53–4.12)
Glucose T = 0 min	1	3.47 (1.53–7.87)	1.35 (1.08–1.69)	0.89 (0.70–1.13)	0.93 (0.49–1.77)
	2	2.73 (1.18–6.30)	1.30 (1.03–1.63)	0.83 (0.65–1.07)	0.75 (0.40–1.42)
	3	2.72 (1.05–7.02)	1.29 (1.00–1.67)	0.83 (0.63–1.08)	0.87 (0.40–1.87)
Glucose T = 120 min	1	6.16 (1.66–22.78)	1.30 (0.99–1.70)	0.81 (0.63–1.06)	1.56 (0.58–4.22)
	2	4.76 (1.20–18.92)	1.22 (0.91–1.62)	0.73 (0.56–0.96)	1.17 (0.44–3.09)
	3	4.34 (1.07–17.73)	1.18 (0.88–1.59)	0.73 (0.55–0.96)	1.63 (0.52–5.14)

Data analysis by logistic regression; odds ratios expressed per standard deviation (SD) increase of independent variable; plasma dicarbonyl and glucose levels LN-transformed prior to analyses; Model (M) 1: adjusted for age, gender, glucose metabolic status; Model 2: model 1 + adjusted for total-to-HDL-C ratio, triglycerides, lipid-modifying treatment, smoking status, systolic blood pressure, blood-pressure-lowering treatment, body mass index, previous cardiovascular disease, glucose-lowering treatment; Model 3: model 2 + adjusted for HbA_{1c}; eGFR: estimated glomerular filtration rate; MGO: methylglyoxal; GO: glyoxal; 3-DG: 3-deoxyglucosone; HDL-C: high-density lipoprotein cholesterol.

As for 3-DG, an association was observed between fasting and post-OGTT plasma 3-DG levels and greater odds of having macroalbuminuria. On the other hand, this association was not observed for the presence of albuminuria (Table 3, models 1–3) and, when UAE was analyzed on a continuous scale, this association was only present for fasting plasma levels of GO. In addition, higher post-OGTT levels of 3-DG, but not fasting plasma 3-DG levels, were associated with higher eGFR values (Table S1).

Moreover, plasma glucose levels, but not a decreased eGFR or the presence of retinopathy were, in the present analyses, associated with significantly greater odds of having (macro)-albuminuria (Table 3, models 1–3). Finally, no consistent inter-

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Table 4
Associations between plasma levels of dicarbonyl and markers of microvascular dysfunction.

	Model	Retinal arterial dilatation	Retinal venular dilatation	Heat-induced skin hyperaemia	Microvascular endothelial dysfunction score
MGO T = 0 min	1	−0.03 (−0.07 to 0.02)	−0.03 (−0.08 to 0.01)	0.04 (−0.01 to 0.09)	0.04 (0.00 to 0.07)
	2	−0.03 (−0.07 to 0.02)	−0.03 (−0.07 to 0.02)	0.04 (−0.01 to 0.10)	0.03 (0.00 to 0.07)
	3	−0.02 (−0.07 to 0.02)	−0.03 (−0.07 to 0.02)	0.05 (−0.01 to 0.10)	0.03 (−0.01 to 0.06)
MGO T = 120 min	1	−0.03 (−0.08 to 0.02)	−0.02 (−0.08 to 0.03)	−0.03 (−0.08 to 0.02)	0.04 (0.00 to 0.08)
	2	−0.03 (−0.08 to 0.02)	−0.02 (−0.07 to 0.03)	−0.03 (−0.08 to 0.02)	0.03 (−0.01 to 0.07)
	3	−0.03 (−0.08 to 0.03)	−0.02 (−0.07 to 0.04)	−0.03 (−0.08 to 0.03)	0.03 (−0.02 to 0.07)
GO T = 0 min	1	0.02 (−0.03 to 0.06)	−0.05 (−0.09 to −0.01)	−0.06 (−0.11 to 0.01)	−0.01 (−0.04 to 0.03)
	2	0.02 (−0.03 to 0.06)	−0.06 (−0.10 to −0.01)	−0.05 (−0.10 to 0.00)	0.03 (−0.01 to 0.06)
	3	0.02 (−0.03 to 0.06)	−0.06 (−0.10 to −0.01)	−0.05 (−0.10 to 0.00)	0.03 (−0.01 to 0.06)
GO T = 120 min	1	0.06 (−0.09 to 0.19)	−0.02 (−0.06 to 0.19)	0.03 (−0.02 to 0.07)	−0.03 (−0.07 to 0.01)
	2	0.06 (−0.08 to 0.20)	−0.03 (−0.07 to 0.02)	0.03 (−0.02 to 0.08)	−0.01 (−0.04 to 0.03)
	3	0.07 (−0.08 to 0.21)	−0.03 (−0.07 to 0.02)	0.03 (−0.02 to 0.08)	−0.01 (−0.05 to 0.03)
3-DG T = 0 min	1	−0.07 (−0.13 to −0.01)	−0.03 (−0.09 to 0.03)	0.14 (0.07 to 0.21)	0.07 (0.02 to 0.12)
	2	−0.07 (−0.14 to −0.01)	−0.02 (−0.09 to 0.04)	0.13 (0.06 to 0.21)	0.02 (−0.03 to 0.07)
	3	−0.07 (−0.14 to 0.00)	−0.02 (−0.09 to 0.05)	0.16 (0.09 to 0.24)	0.01 (−0.05 to 0.06)
3-DG T = 120 min	1	0.01 (−0.07 to 0.09)	−0.03 (−0.11 to 0.05)	0.01 (−0.07 to 0.09)	0.14 (0.08 to 0.20)
	2	0.02 (−0.07 to 0.10)	−0.02 (−0.10 to 0.07)	0.02 (−0.07 to 0.10)	0.08 (0.02 to 0.15)
	3	0.03 (−0.06 to 0.11)	−0.01 (−0.10 to 0.07)	0.03 (−0.06 to 0.11)	0.08 (0.01 to 0.14)
Glucose T = 0 min	1	−0.05 (−0.13 to 0.02)	−0.06 (−0.14 to 0.01)	0.00 (−0.09 to 0.09)	0.12 (0.07 to 0.18)
	2	−0.06 (−0.14 to 0.02)	−0.06 (−0.14 to 0.01)	−0.01 (−0.10 to 0.07)	0.06 (0.00 to 0.11)
	3	−0.05 (−0.13 to 0.03)	−0.06 (−0.14 to 0.02)	0.01 (−0.08 to 0.11)	0.04 (−0.02 to 0.10)
Glucose T = 120 min	1	0.06 (−0.03 to 0.14)	0.00 (−0.09 to 0.08)	0.06 (−0.03 to 0.14)	0.13 (0.06 to 0.19)
	2	0.06 (−0.02 to 0.15)	0.00 (−0.08 to 0.09)	0.06 (−0.02 to 0.15)	0.07 (0.01 to 0.14)
	3	0.07 (−0.01 to 0.16)	0.01 (−0.08 to 0.10)	0.08 (−0.01 to 0.16)	0.06 (0.00 to 0.13)

Data analysis by linear regression; regression coefficients expressed as 1 standard deviation (SD) difference in outcome per SD increase of plasma dicarbonyl or glucose; skin hyperaemia and plasma glucose and dicarbonyl levels LN-transformed prior to analyses; Model 1: adjusted for age, gender, glucose metabolic status; Model 2: model 1 + adjusted for total-to-HDL-C ratio, triglycerides, lipid-modifying treatment, smoking status, systolic blood pressure, blood-pressure-lowering treatment, body mass index, previous cardiovascular disease, glucose-lowering treatment; Model 3: model 2 + adjusted for HbA_{1c}; MGO: methylglyoxal; GO: glyoxal; 3-DG: 3-deoxyglucosone; HDL-C: high-density lipoprotein cholesterol.

actions with either gender or glucose metabolic status were found for these associations (data not shown).

Fasting and post-OGTT plasma dicarbonyls and plasma microvascular ED, retinal arteriolar/venular average dilatation and heat-induced skin hyperaemia

Whether plasma dicarbonyls are associated with markers of microvascular ED and activation was also investigated. After adjusting for gender, age and glucose metabolic status, higher fasting and post-OGTT plasma MGO levels were associated with higher plasma microvascular ED scores (Table 4, model 1), although this association was attenuated and no longer significant after further adjusting (Table 4, models 2–3). On analyzing microvascular endothelial plasma markers separately, the main findings were an association between higher plasma MGO and plasma sE-selectin levels (Table S2; see supplementary materials associated with this material online). In addition, no associations were noted between fasting and post-OGTT plasma MGO levels, retinal arteriolar or venular dilatation and heat-induced skin hyperaemia (Table 4), nor were there any significant associations between fasting and post-OGTT plasma GO levels and any microvascular endothelial (dys)function markers (Table 4).

Higher fasting plasma 3-DG levels were associated with higher plasma microvascular ED scores after adjusting for gender, age and glucose metabolic status (Table 4, model 1), although this association was no longer statistically significant after additional adjustments (Table 4, models 2–3). In line with this, higher post-OGTT plasma 3-DG levels were associated with higher plasma microvascular ED scores (Table 4, model 1), an association that was attenuated, but remained statistically significant, after further adjustments (Table 4, models 2–3). Surprisingly, higher fasting plasma 3-DG levels were associated with greater heat-induced skin hyperaemia responses even in the fully adjusted model (Table 4, models 1–3), a finding that did not apply to post-OGTT plasma 3-DG levels. Furthermore, higher fasting and post-OGTT plasma glucose levels were associated with significantly higher plasma microvascular ED scores (Table 4, models 1–3). Finally, no consistent interactions were observed with either gender or glucose metabolic status for these associations (data not shown).

Discussion

Our present study has found, in a population-based observational cohort study enriched by the inclusion of T2DM patients, that higher fasting and post-OGTT plasma MGO levels are associated with markers of CKD and the presence of retinopathy, but not with prior CVD, ABI or cIMT. Higher plasma MGO levels were also associated with plasma biomarkers of microvascular ED and sE-selectin in particular. Associations between post-OGTT and fasting plasma MGO levels and disease outcomes appear to be of similar strength. However, no consistent associations with either plasma GO or 3-DG levels were found.

Nevertheless, these present findings expand our previous work by evaluating not only fasting, but also post-OGTT, levels of dicarbonyl stress. This is important as it was previously confirmed that plasma dicarbonyl levels rise rapidly after OGTT or a mixed meal [3,9]. Because post-OGTT rather than fasting plasma glucose levels are more strongly associated with incident CVD [10], our hypothesis was that any associations with vascular disease would also be stronger with post-OGTT compared with fasting dicarbonyl levels. However, our study has found that post-OGTT plasma MGO levels are, in general, no more strongly associated with vascular disease than are fasting plasma MGO levels. Likewise, associations between MGO and albuminuria and a decreased eGFR were apparently attenuated after adjusting for fasting MGO levels. This may have been due to the possibility that post-OGTT levels of plasma dicarbonyls are likely to underestimate the actual peak plasma dicarbonyl levels reached during the day, as studies using

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continuous glucose monitoring imply that intermittent hyperglycaemic spikes are more common than previously thought and are also not consistently reflected by post-OGTT plasma glucose levels [21].

Although it was previously found that higher fasting plasma MGO levels are associated with incident CVD in both T1DM and T2DM [7], our present study could find no such associations with prior CVD or subclinical markers of atherosclerosis. In fact, an earlier report found only prospective associations between plasma MGO levels and cIMT in T2DM [22]. Interestingly, a similar discrepancy was found for plasma AGEs in which only associations with incident CVD [23,24], but not with prior CVD, were found [25]. Therefore, our cross-sectional analyses should be interpreted with caution.

In contrast to our findings for CVD, the present findings confirm associations between higher plasma MGO levels and markers of CKD [7,8], and also demonstrate that these associations not only apply to T1DM and T2DM, but also to prediabetes (IGM) and NGM. The present study mostly included individuals with relatively good metabolic control. This is important as a recent update of the Veterans Affairs Diabetes Trial (VADT) revealed that the legacy effect, often attributed in part to glycation [26], was no longer present in that study [27], leading to speculation that this was partly due to intensified treatment of cardiovascular risk. Yet, our present study shows that, among subjects with a high proportion using antihypertensive, lipid-modifying and glucose-lowering treatments (Table 1), the associations between MGO and markers of CKD nevertheless persist.

Preclinical studies have linked MGO to endothelial activation, impaired vasoreactivity [28–30] and epigenetic changes in endothelial cells [31]. Although the present report suggests an association between higher MGO levels and higher levels of plasma endothelial activation markers, our main finding was an association with sE-selectin, whereas previous animal studies reported increased expression of ICAM and VCAM [29,30]. On the other hand, an earlier study found that plasma MGO levels were associated with sICAM and sVCAM in T1DM [7]. Furthermore, there was no evidence of any associations with other markers of microvascular dysfunction. Although extrapolation should be done with caution among these studies, it could be hypothesized that the type of endothelial injury caused by MGO may differ according to type of diabetes, insulin sensitivity, control of other metabolic risk factors and the vascular bed being studied. This is an important notion, as inhibition of dicarbonyl stress could yield a greater reduction of vascular complications, depending on the type of diabetes and severity of metabolic dysregulation.

The major strengths of the present study include its large sample size, state-of-the-art equipment for estimating plasma dicarbonyl levels and in-depth phenotyping of vascular function. However, there are also several limitations. First of all, due to the cross-sectional nature of the study, no causality can be inferred for the association between plasma MGO levels and microvascular disease. Although consistent associations of MGO with albuminuria and eGFR were found, the possibility that MGO is a consequence of kidney failure rather than a cause of such failure cannot be excluded [32,33]. In addition, the Maastricht Study mainly includes Caucasians, which should be taken into account when generalizing our present findings. In addition, a questionnaire was used to assess prior CVD, which may have led to an underestimation of the associations with this outcome due to recall bias. However, this may be considered unlikely as no associations with cIMT and ABI were found. Finally, the present study did not take into account all chronic conditions linked to diabetes in which dicarbonyl stress may play a role. It is worth mentioning diabetic neuropathy in particular, as MGO has been identified as a potential driver of that condition [34]. Thus, the present study cannot be used to extrapolate any associations between plasma dicarbonyls and other conditions linked to (pre)diabetes.

Conclusion

The present study has established that higher plasma MGO levels both before and after an OGTT are associated with markers of CKD in subjects with NGM, prediabetes or T2DM. These findings therefore identify MGO as a potential therapeutic target to reduce the burden of CKD in the general population, and highlight the importance of targeting this reactive metabolite with new compounds.

Authors' contributions

NMJH analyzed the data and wrote the manuscript. JLJMS measured dicarbonyls, and wrote and edited the manuscript. MvdW measured dicarbonyls and edited the manuscript. AH, TB, CAW, KDR, MvG, CvdK, NCS, MS, RMAH and CDAS edited the manuscript. CS wrote and edited the manuscript.

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Ethics approval

The Maastricht Study was approved by the institutional medical ethics committee (NL31329.068.10) and Netherlands Health Council under the Dutch Law for Population Studies (permit 131088-105234-PG). All participants gave their written informed consent.

Disclosure of interest

The authors declare that they have no competing interest.

Appendix A. Supplementary data

Supplementary data (Tables S1 and S2) associated with this article can be found, in the online version, at https://doi.org/10.1016/j.diabet.2020.02.002.

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